

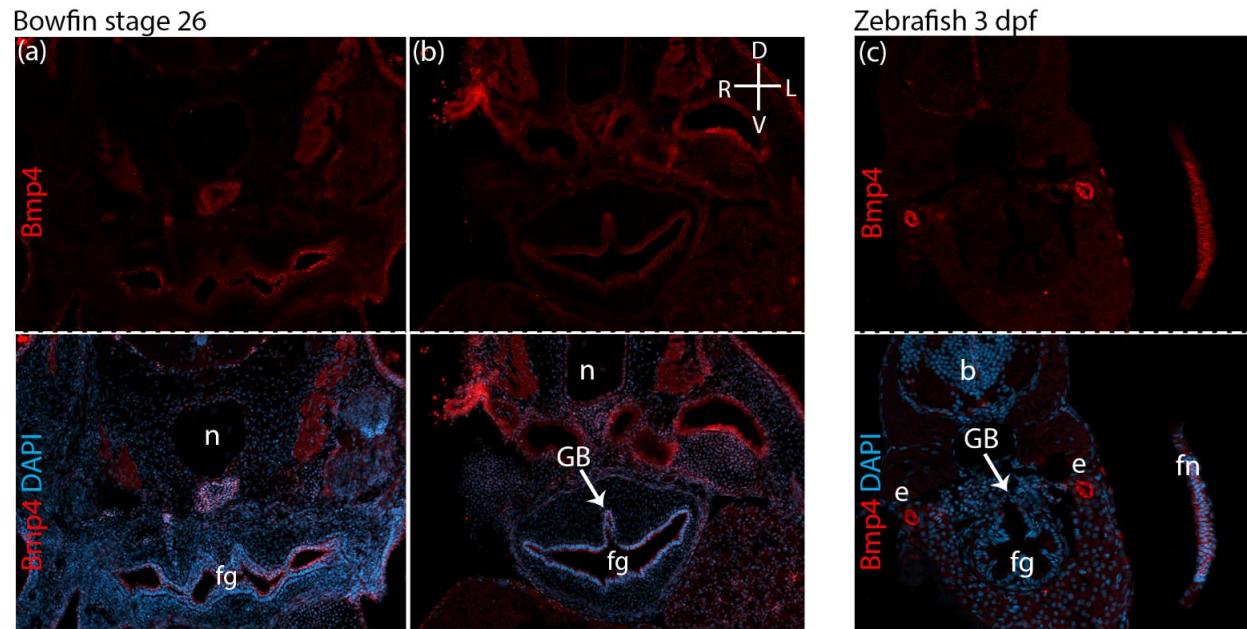
**Figure S1: Bowfin Bmp16 epitope for custom Bmp16 antibody design.** The alignment of bowfin Bmp16, Bmp4, and Bmp2 proteins shows conservation between the protein sequences only at the C-terminal. The Bmp16 epitope (highlighted in yellow) used to design the Bmp16 antibody is in a region of the protein that is distinct from Bmp4 and Bmp2. Therefore, the bowfin Bmp16 antibody should not cross-react with either Bmp4 or Bmp2.

BMP16	-----	0
BMP4	-----	0
BMP2	MSFRCLITYCVDVKSSMSIVKPTAVLLTLLFCVACGGQVGDVPKQERLFLSALGLSSRPK	60
BMP16	-----	0
BMP4	-----	0
BMP2	AEGRFPVPSLLWKIFKRKTIGKEAVDPEHDPC TVSEFKVRGNIVRFIQDQGRLLPVFHRQ	120
BMP16	-----	0
BMP4	-----	0
BMP2	CPSCIERHLYFNMSVLQDVEQLTLAQLEVTFNQDLHRLPRHADLFTMHLYKVMKTTLKGV	180
BMP16	-----	0
BMP4	-----	0
BMP2	KHQSNRKLLLQSFSQLI PGSITLNLTGMAEIWRKPGKNFGLVLTIQRSAPIDNTDPVRLM	240
BMP16	-----	0
BMP4	-----	0
BMP2	NLQNDVEHSLVGHFPFYASLVVVSLNPLQCRSRRRRSAYYFPVTPSNVCKARRLYIDFK	300
BMP16	-----	0
BMP4	-----	0
BMP2	DVGWQDWIIAPQGYMANYCKGECPFPLSESLngTNHAILQTLVHSFDPTGTQPCCVPVR	360
BMP16	-----	0
BMP4	-----	0



BMP16 CCVPTSLSPIAMLYLDQHDRVVLKNYQDMVVEGCGCR 416  
BMP4 CCVPTELSAISMLYLDEHDKVVLKNYQEMVVEGCGCR 401  
BMP2 CCVPTELSAISLLYLDYEKVILKNYQDMAVEGCGCR 835  
\*\*\*\*\*.\* \* \* : \* : \* : \* : \* : \* . \*\*\*\*\*

**Figure S2: *Bmp4* expression in bowfin and zebrafish:** For each column, top panels show *Bmp4* expression alone in bright red, and bottom panels show *Bmp4* expression in bright red overlain with DAPI in blue. All panels are transverse sections. **a)** *Bmp4* expression in bowfin at stage 26 in the foregut (fg), anterior to the gas bladder bud. *Bmp4* is expressed throughout the foregut anterior to the site of gas bladder budding. **b)** *Bmp4* expression in the gas bladder (GB) of bowfin at stage 26, right after budding. At the site of budding, *Bmp4* expression is absent from the foregut and the gas bladder bud. **c)** *Bmp4* expression in zebrafish at 3 dpf. *Bmp4* is not expressed in the foregut or the gas bladder but is expressed in the inner ear (e) and fin (fn). Abbreviations: b, brain; n, notochord.



**Figure S3: Bmp16 Immunofluorescence negative control in mouse.** For each column, top panels show Bmp16 staining alone in bright red. Middle panels show Bmp16 expression in red overlain with autofluorescence in bright green. Bottom panels show Bmp16 expression in red overlain with DAPI staining of the nuclei in blue. All panels are transverse sections of mouse at E12.5. **a)** Bmp16 expression in an anterior region of the esophagus (e) and trachea (t). **b)** Bmp16 expression in a posterior region of the esophagus and trachea. The absence of bright red staining illustrates the absence of Bmp16 expression in mouse tissues, as expected given that the Bmp16 gene is absent in mammalian genomes. The absence of bright red staining also indicates that the bowfin specific anti-Bmp16 antibody is not cross reacting with Bmp4 or Bmp2. Abbreviations: e, esophagus; t, trachea; gr, laryngo-tracheal groove.

